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Probes for Quantitative Optical and Electron Microscopy

Our center is directed towards the parallel development of new fluorescent probes and imaging methodologies to permit quantitative single-molecule sensitivity in living cell systems. Because we have the long-term goal of imaging of single molecules within living tissues and whole animals, we have directed our efforts towards the red and infra-red portion of the spectrum. On the measurement side, we have used fluorescence imaging by wide-field, total internal reflection fluorescence (TIRF) microscopy, deconvolution, confocal, and multi-photon excitation microscopy, along with lifetime and spectral contrast modes. For single molecule resolution, both TIRF and fluorescence correlation spectroscopy have proven most useful. We have also been developing STEM, EELS, and cathodal luminescence as novel high resolution contrast approaches.

We have continued to use three general approaches for creating labels that are useful for both optical and electron microscopy: genetically-encoded proteins, lanthanide chelates, and quantum dots. We have developed appropriate ligands to compare each of these approaches for imaging of a plasma membrane surface target (using the angiotensin receptor as our model system) and an intracellular target (using the mitochondrial peripheral-type benzodiazepine receptor (PBR) as the model system). For the genetic labeling approach, we have been using a newly developed red fluorescent protein with increased quantum efficiency, and we have made progress using photoswitchable dyes for a photochromic FRET approach. For the lanthanide chelates, we have created several new antenna complexes that have a high yield of lanthanide excitation, and are appropriate for both one-photon and two-photon excitation. We have begun using the short polypeptide chelators for genetic lanthanide labeling, but these will need to be modified to add a high-efficiency antenna before they are useful for luminescence imaging in cells. However, a GFP:chelate construct can be used for fluorescence imaging of the GFP followed by EM imaging of the lanthanide. We have also shown that these lanthanide chelates are electron-dense and can be detected by EM without further contrast enhancement. For the quantum dots, we have shown their efficacy for single receptor imaging on the surface of live cells and have developed new surface coatings that allow creation of high-affinity labels. In addition, the new small, white-light quantum dots were created in an effort to make smaller structures for intracellular imaging. While these dots each exhibit a broad fluorescence spectrum and thus are not useful for cellular imaging, they will have impact in many others areas of technology. We are continuing our efforts to make NIR quantum dots using doped CdSe nanoclusters to achieve high quantum efficiency and low background optical imaging in live cells.

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